

# Refining the conformational ensembles of flexible proteins using simulation-guided spectroscopy

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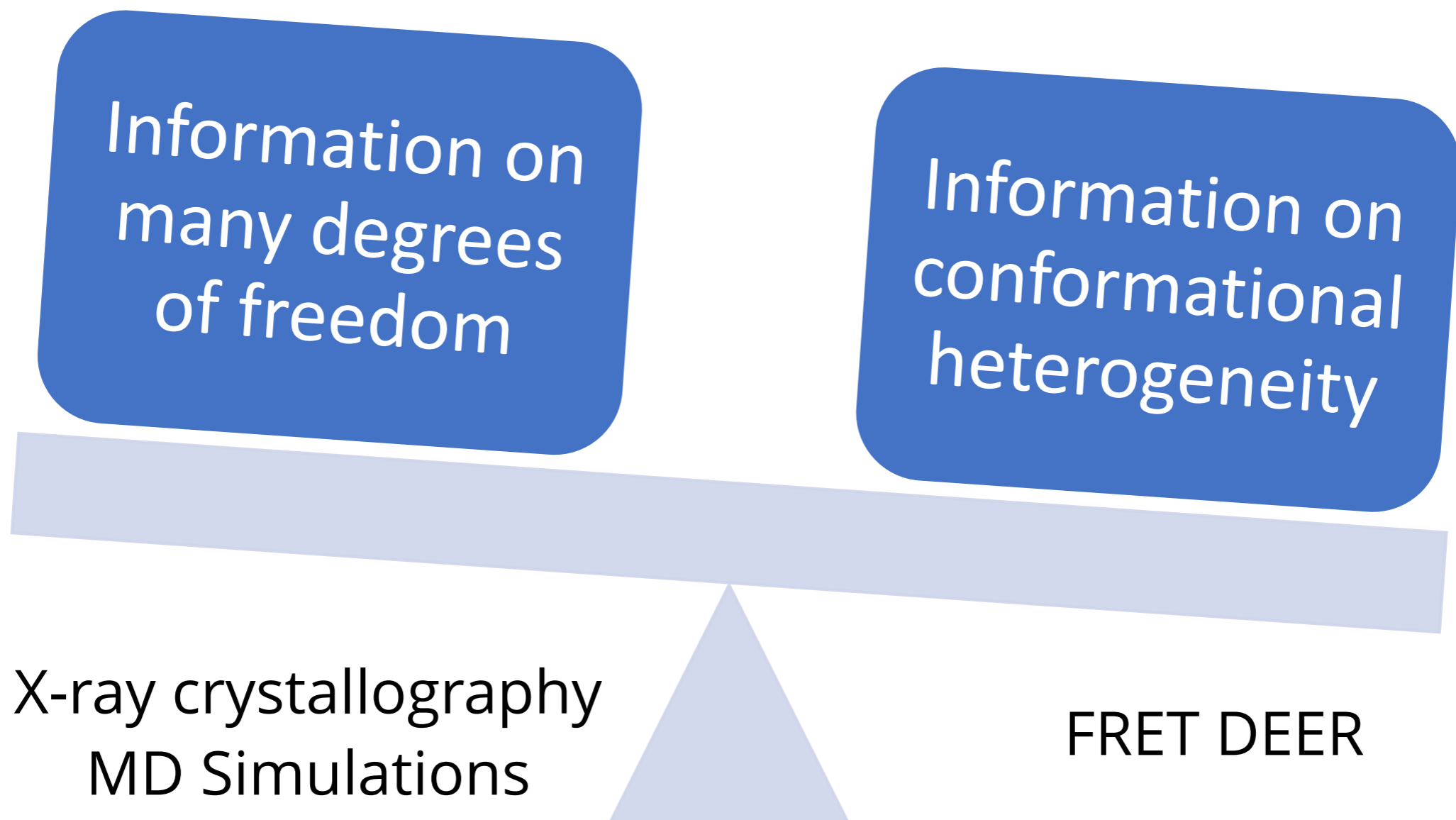
Blue Waters Symposium  
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# Acknowledgements

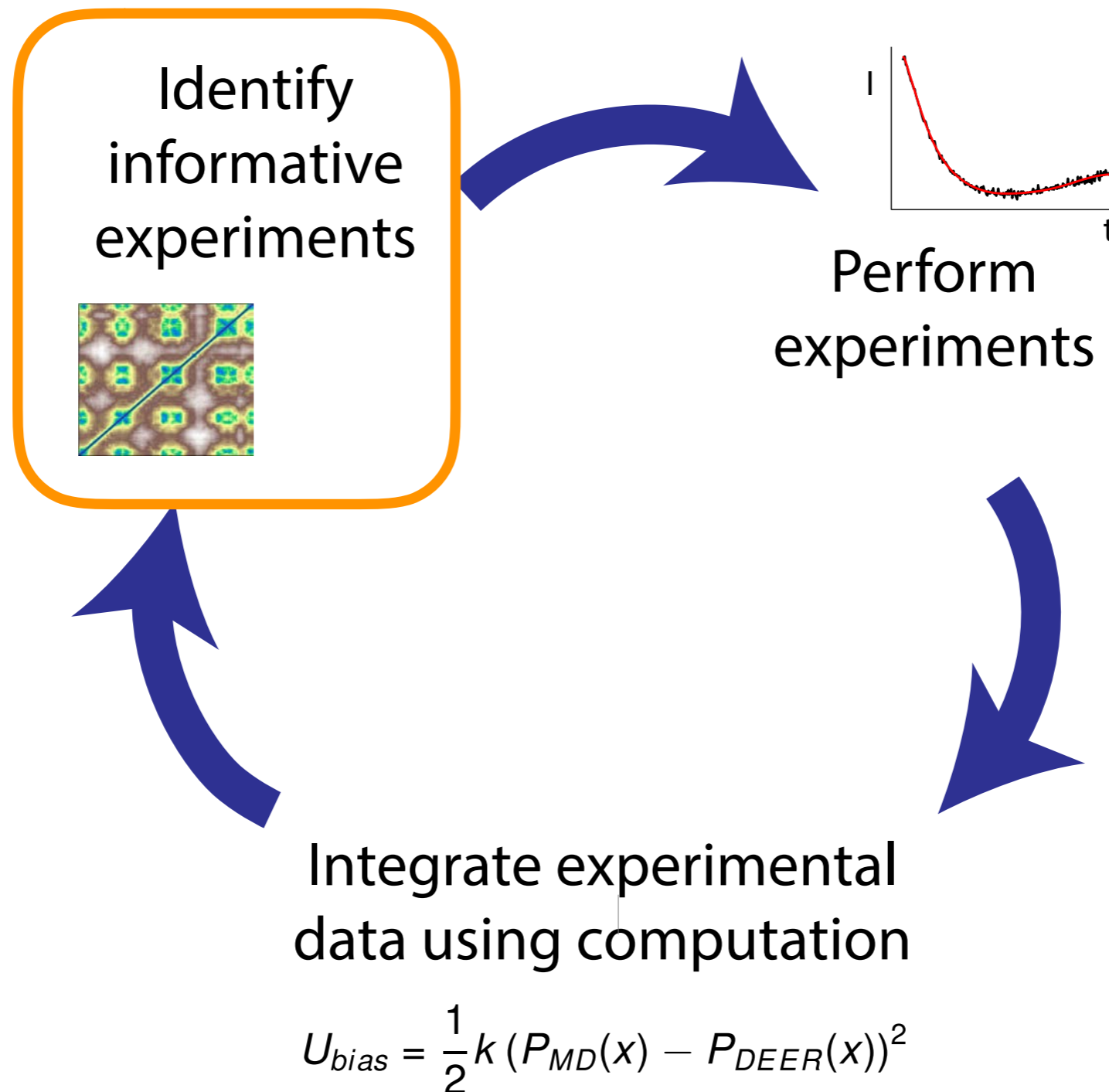
- Kasson Lab
  - Peter Kasson, MD, PhD
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Flexible proteins are hard to study because many states contribute to their conformational ensembles



# Iterative refinement leverages both computation and experiment

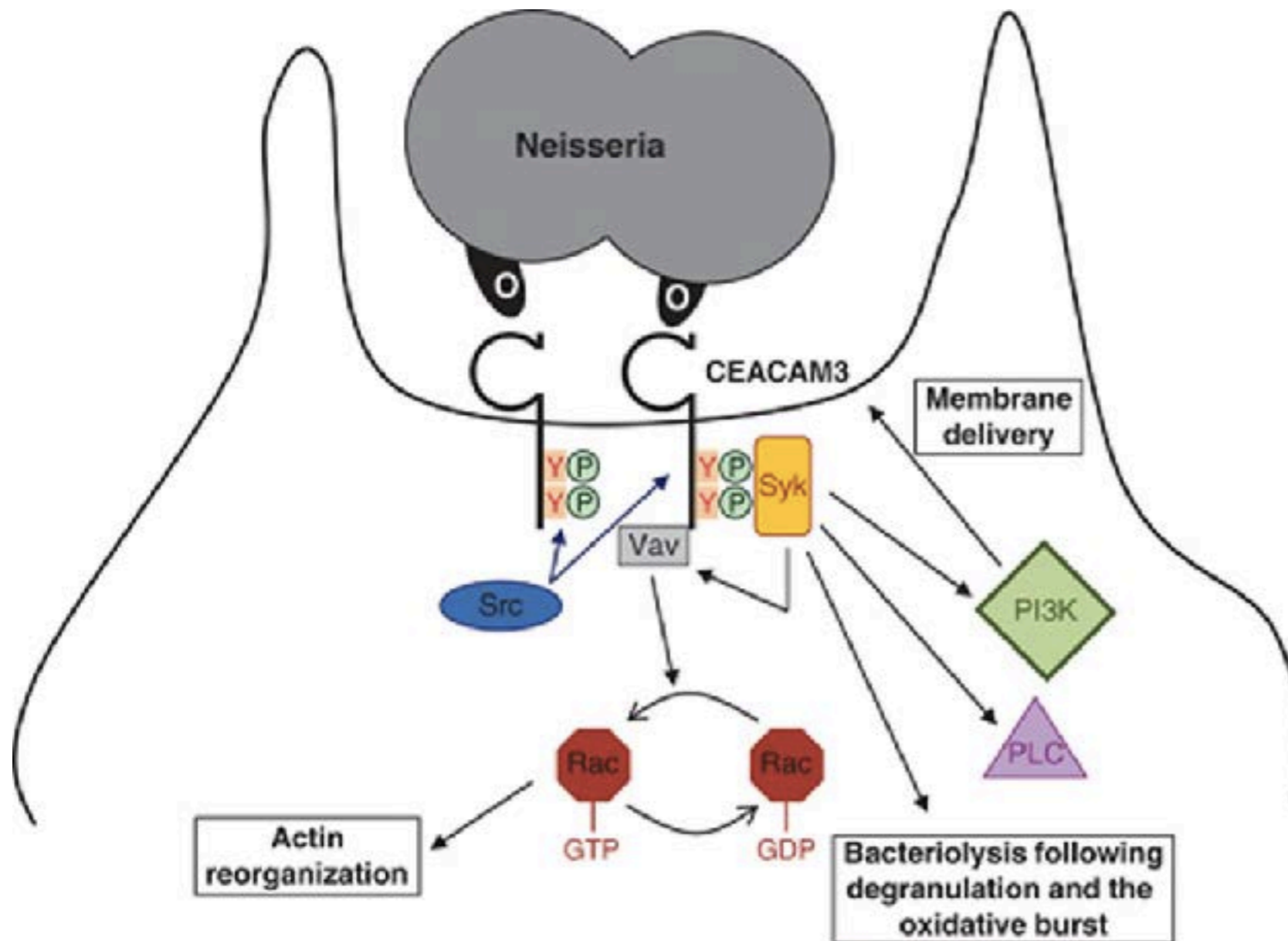


# Opa-CEACAM interaction

CEACAM1

N-terminal Ig Domain

PDB ID: 2GK2

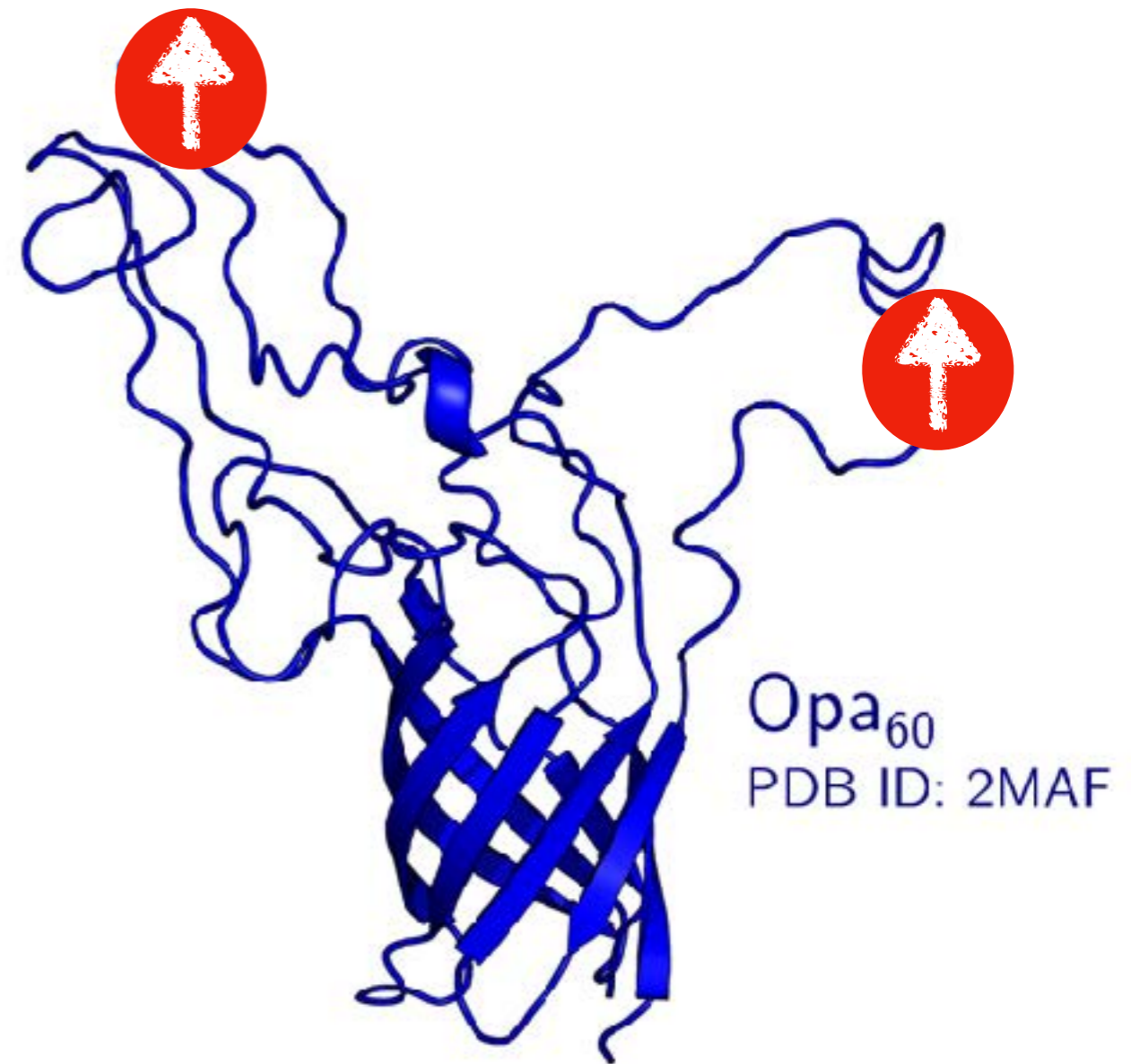


Opa<sub>60</sub>

PDB ID: 2MAF

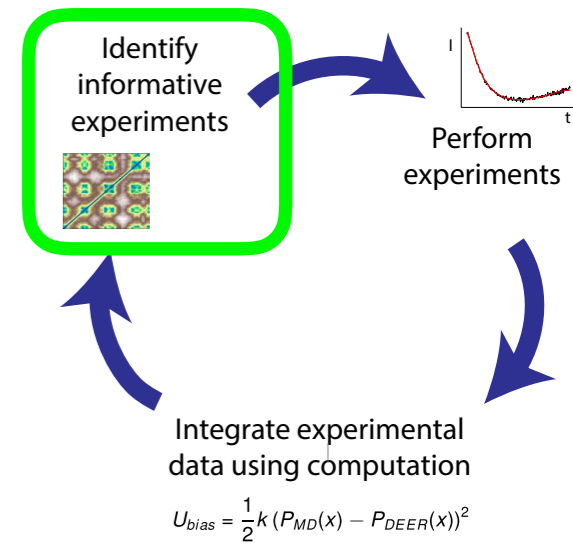
# How we study binding: DEER

- Double electron-electron resonance (DEER) provides distance distributions between pairs of spin-labeled amino acids
- Great! Captures conformational heterogeneity of Opa ensemble
- **Problem:** DEER only reports on a few DOF in the system, but we need on the order of ~10,000 measurements



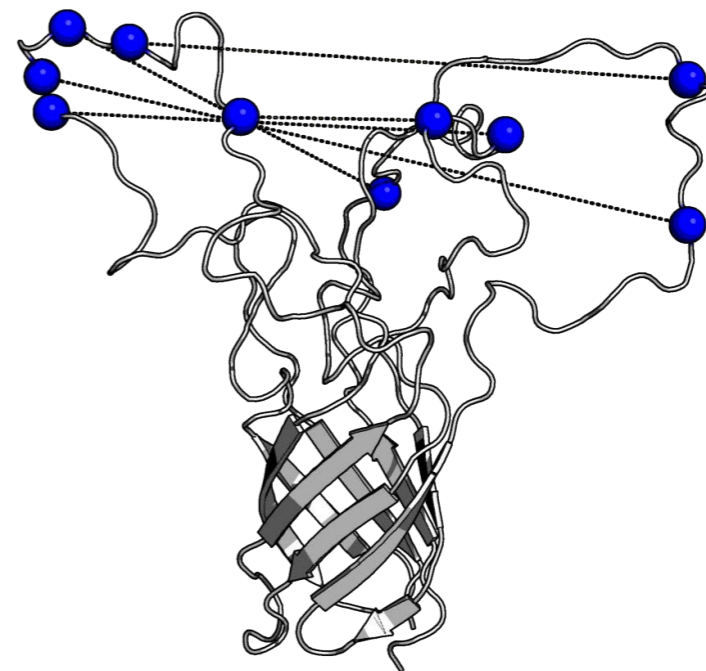
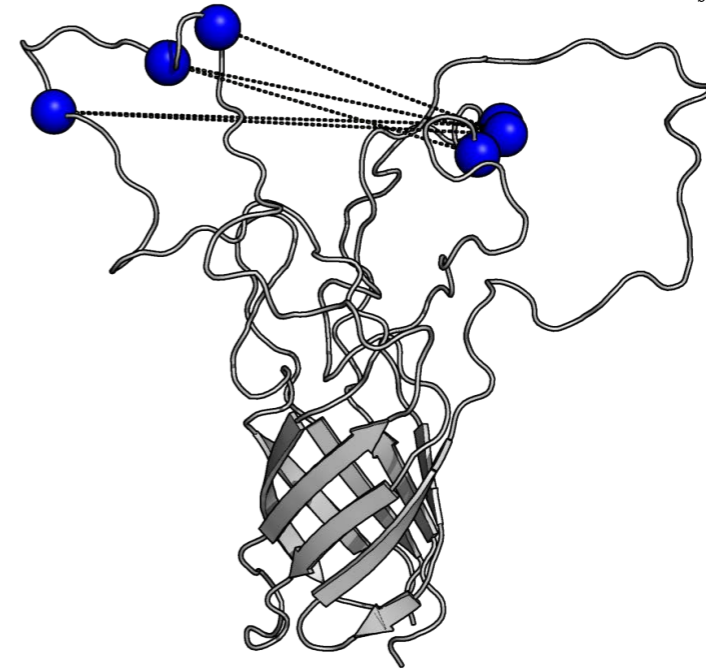
# mRMR Algorithm

(maximum-relevancy, minimum redundancy)

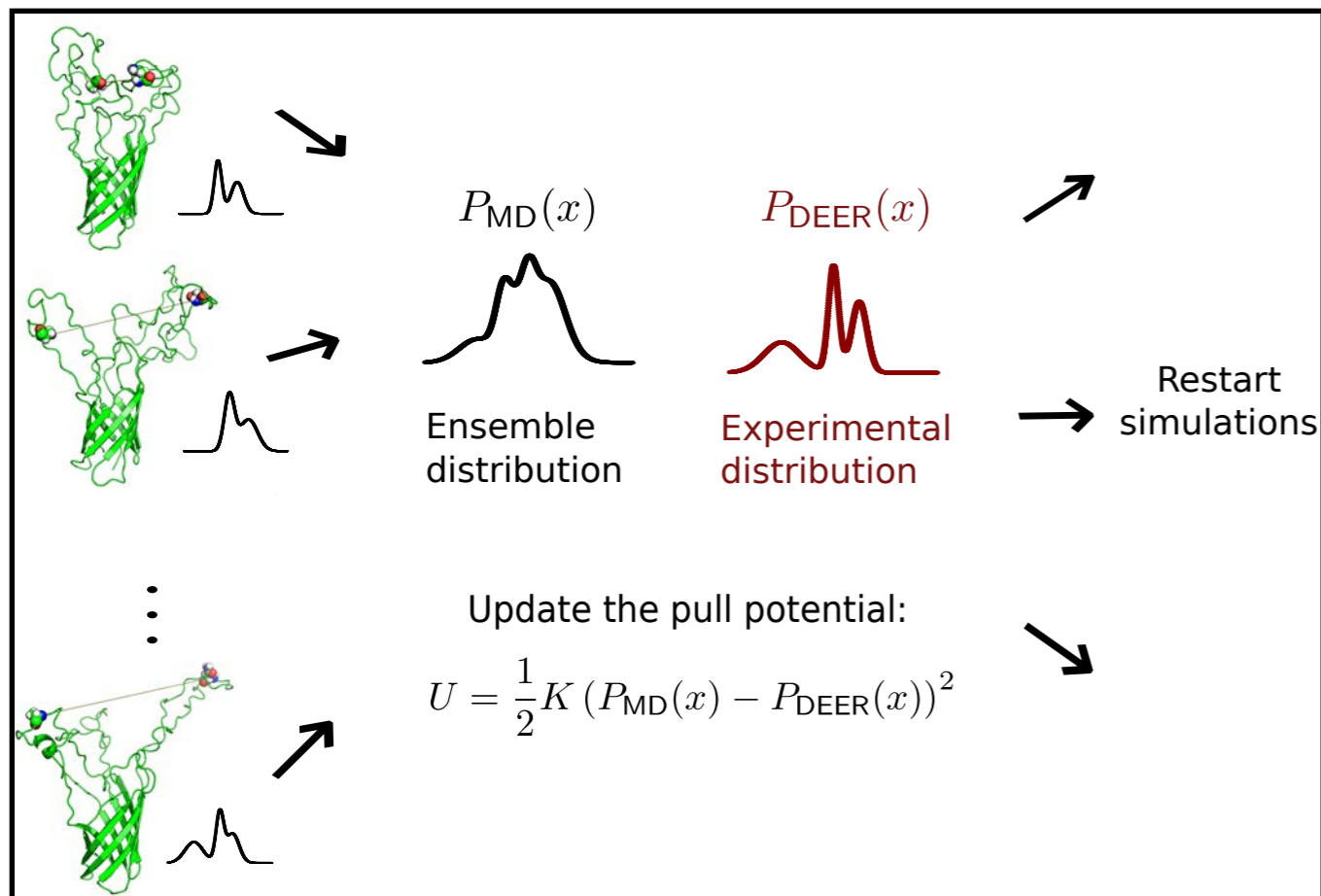
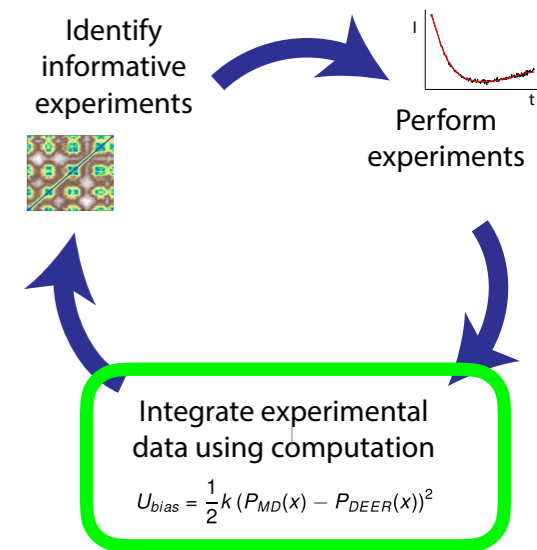


We want two things from our pairs:

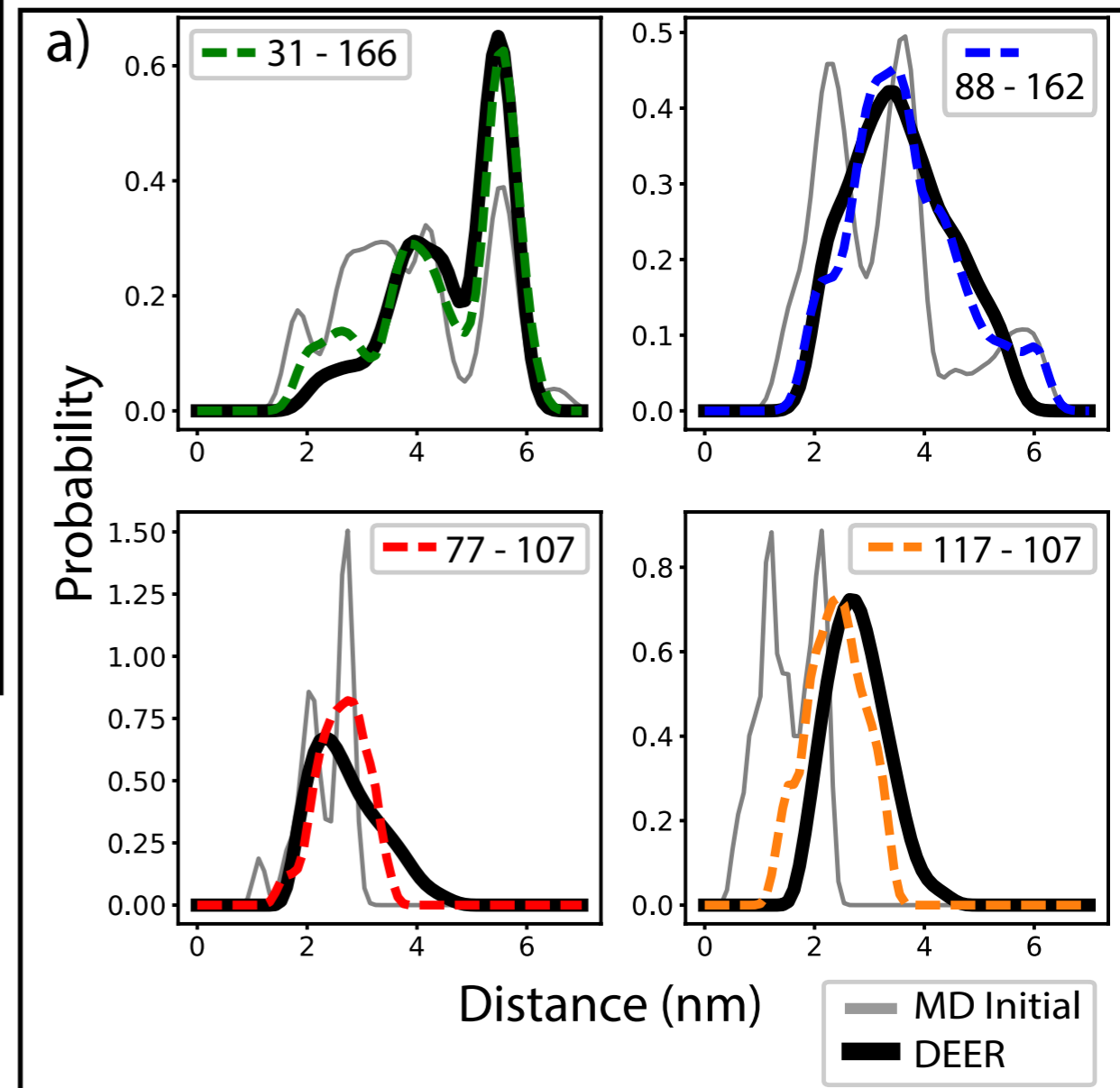
1. maximally informative on the conformational ensemble
2. are minimally redundant with each other



# Integration of spectroscopic data

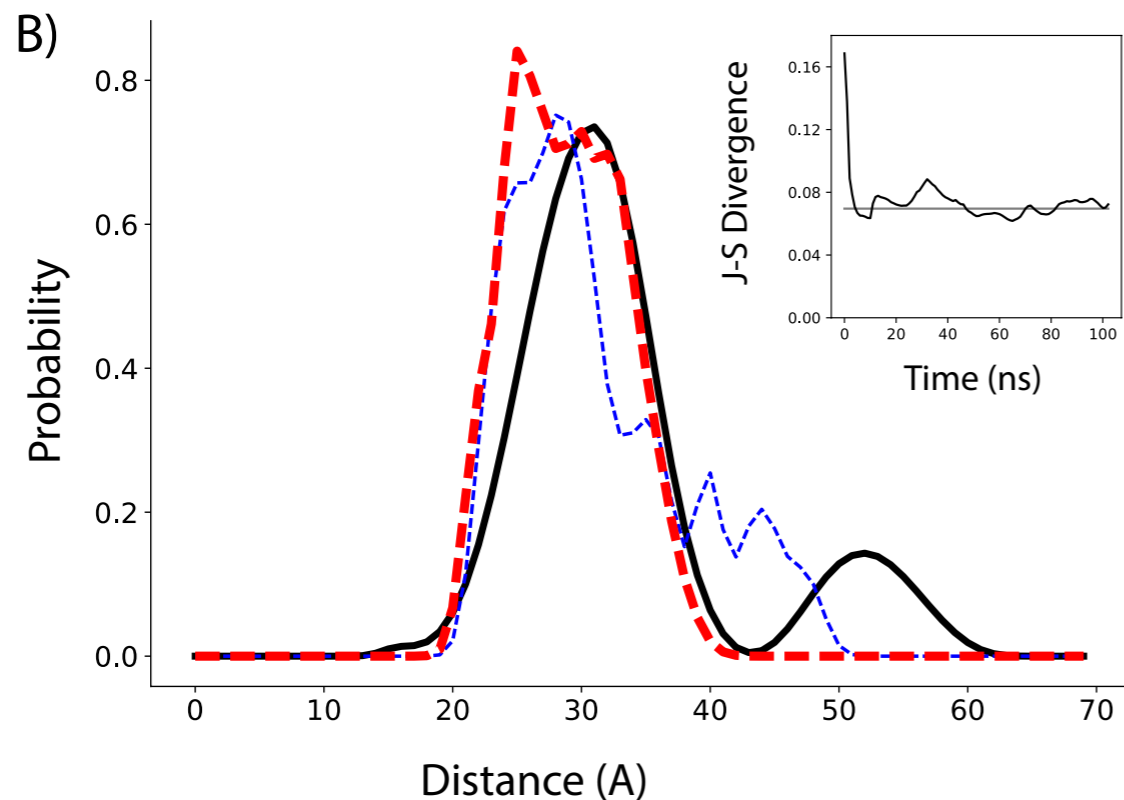
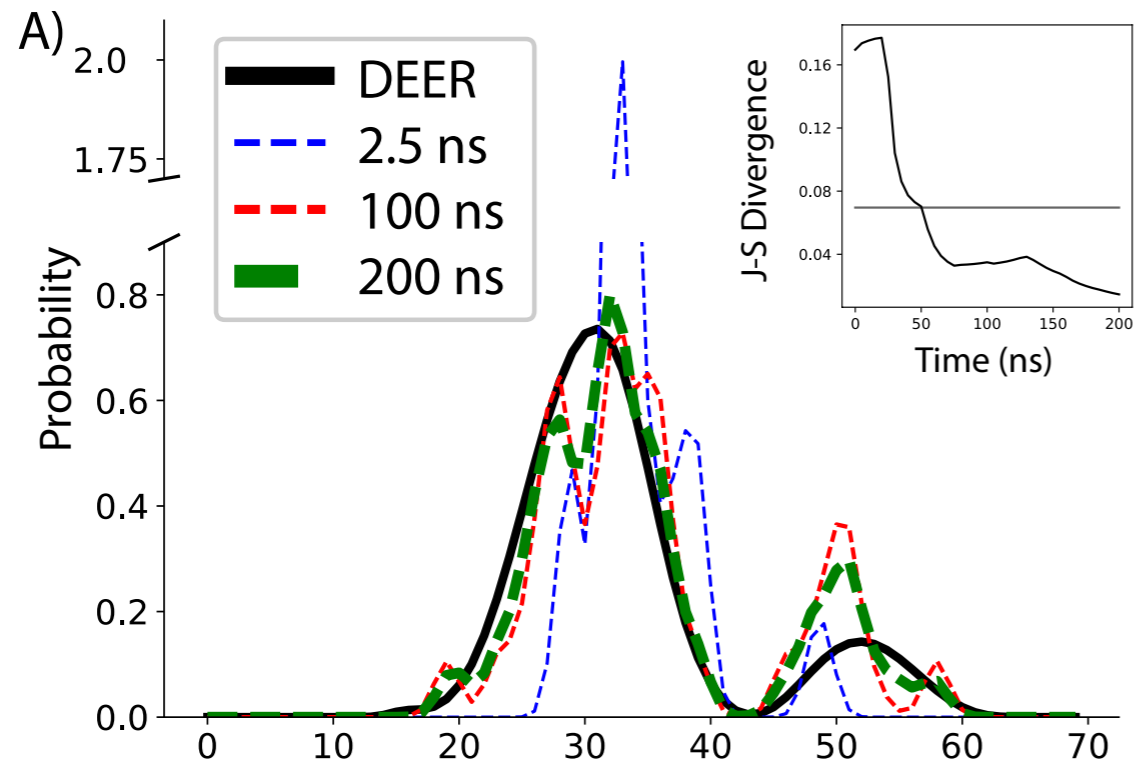
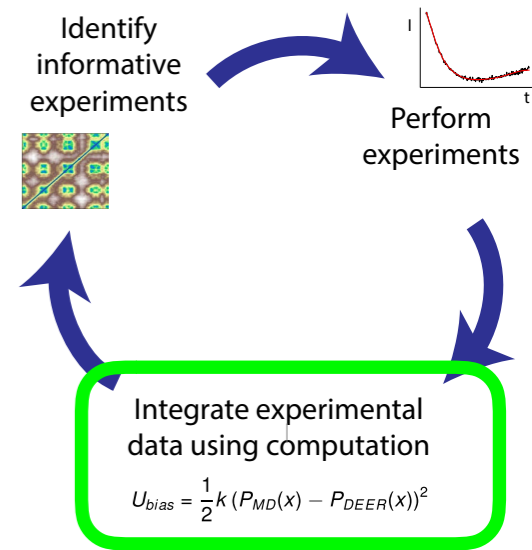


After 100 ns of simulation per ensemble member (2  $\mu$ s aggregate), approximately converge to target





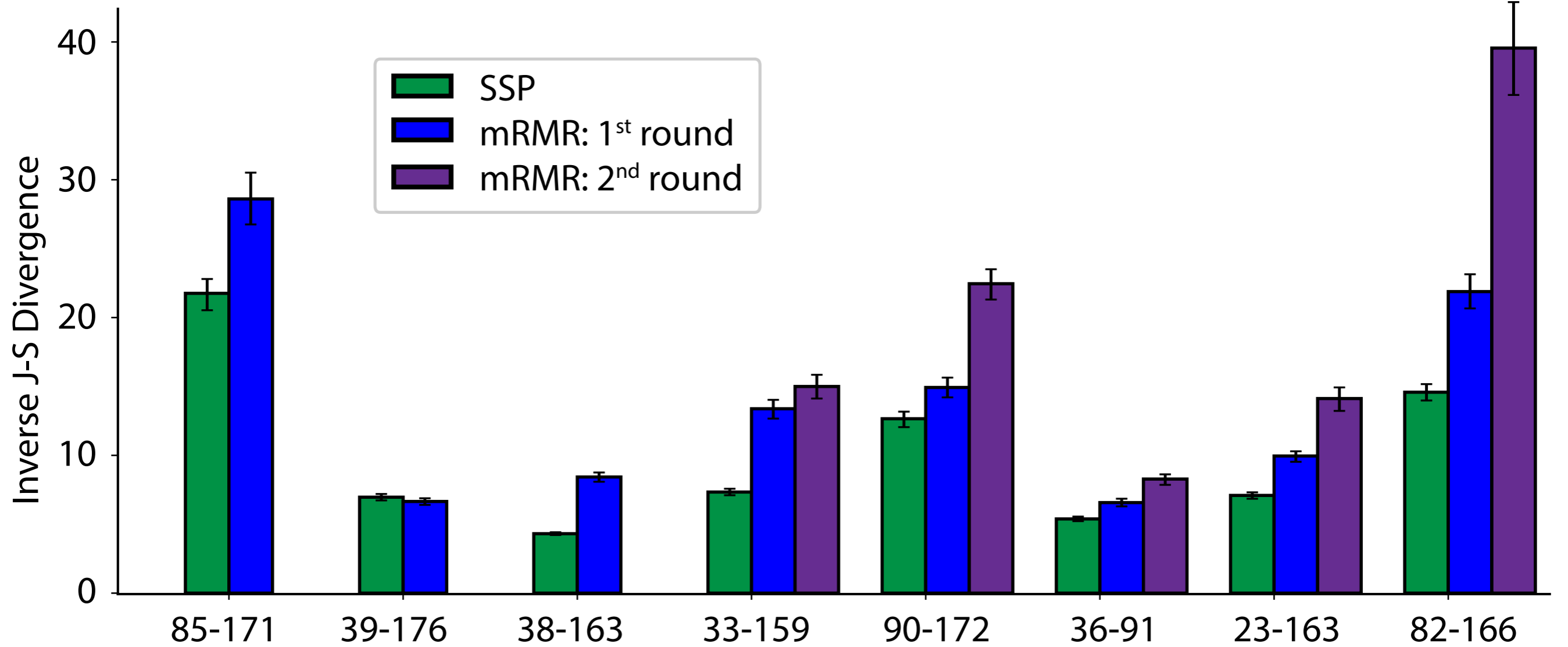
# As a teaser...



Current method not robust enough to handle well-separated probability modes

Come see my poster tonight for more details!

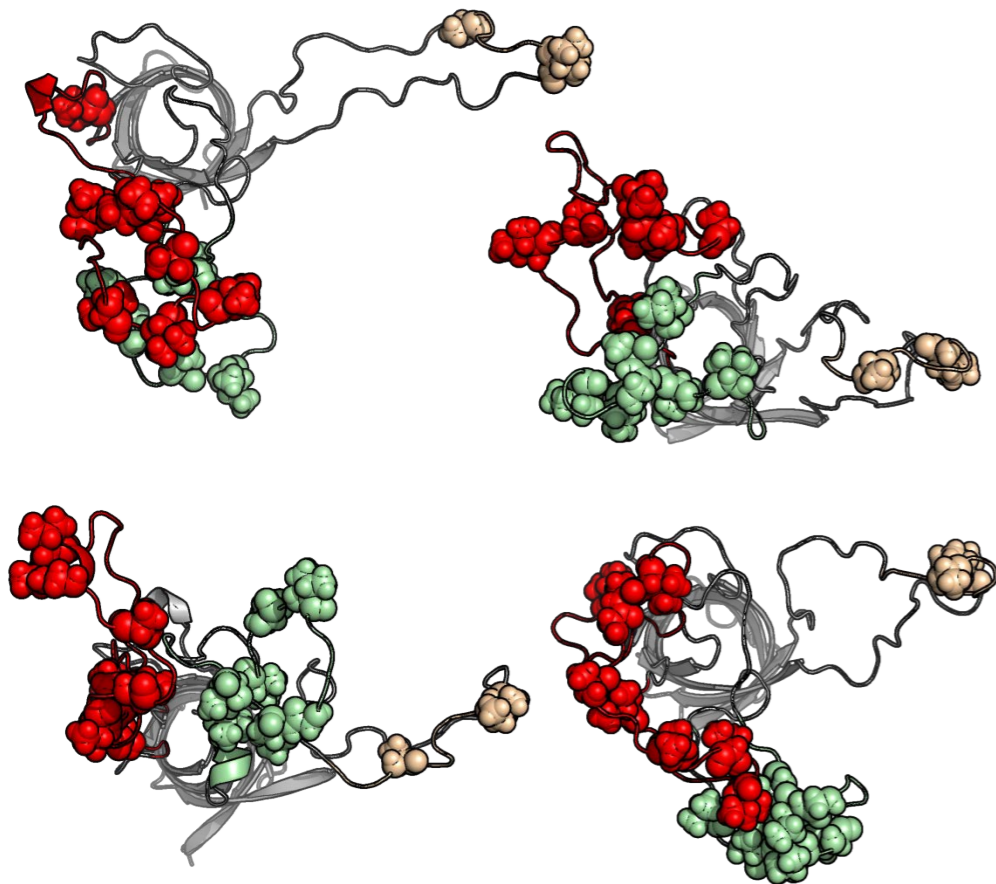
# How well-refined are the resulting ensembles?



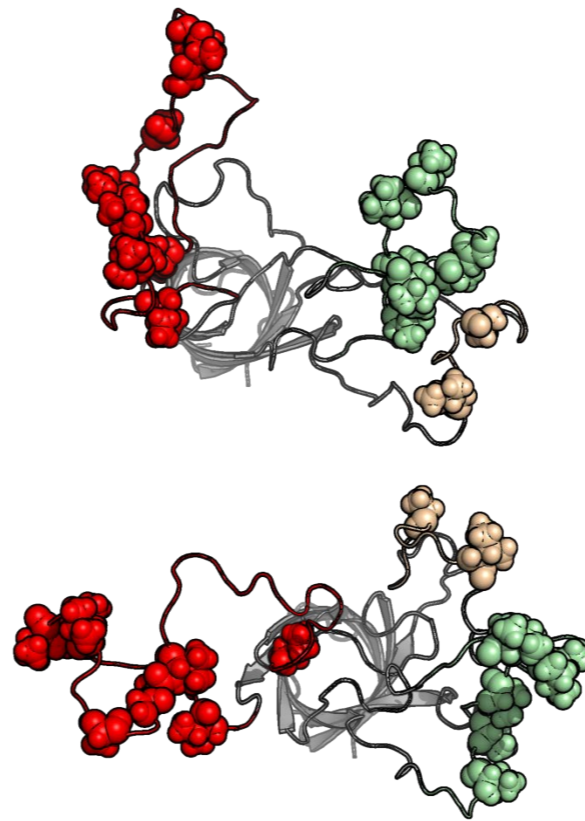
# Two rounds of simulation-guided spectroscopy generate a hypothesis for CEACAM engagement

Opa loops have three regions of high variability: SV (tan), HV1 (green), HV2 (red)

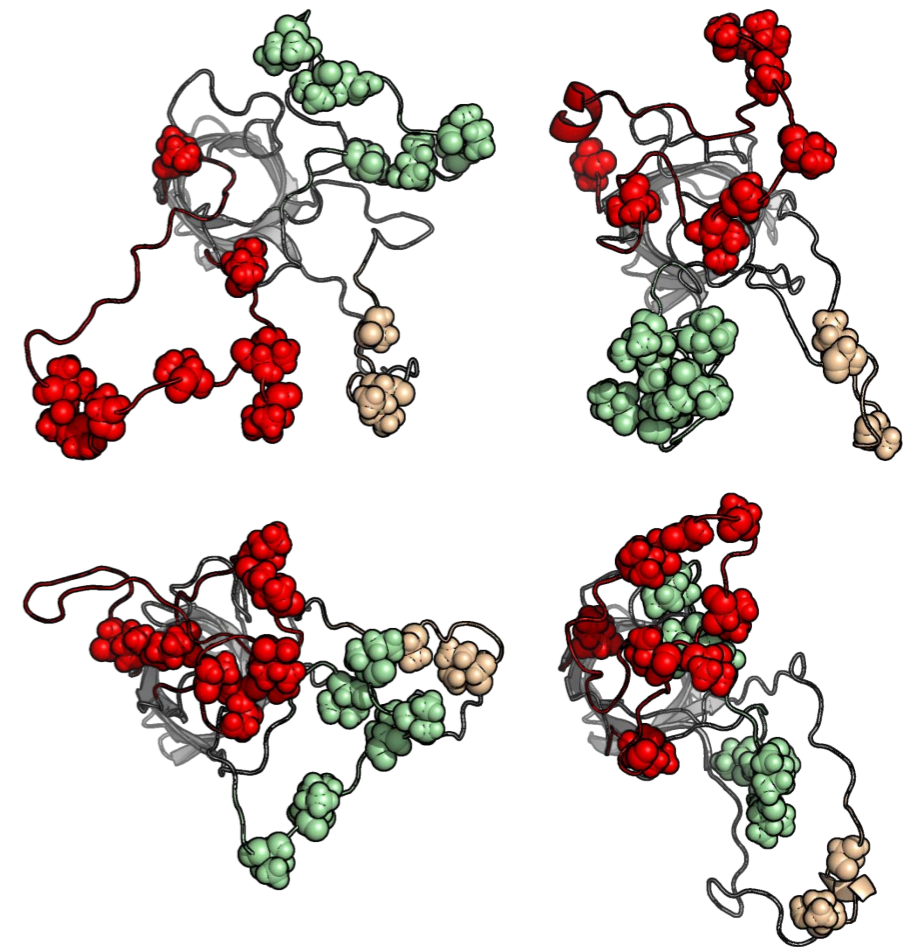
SV extended: 40 %



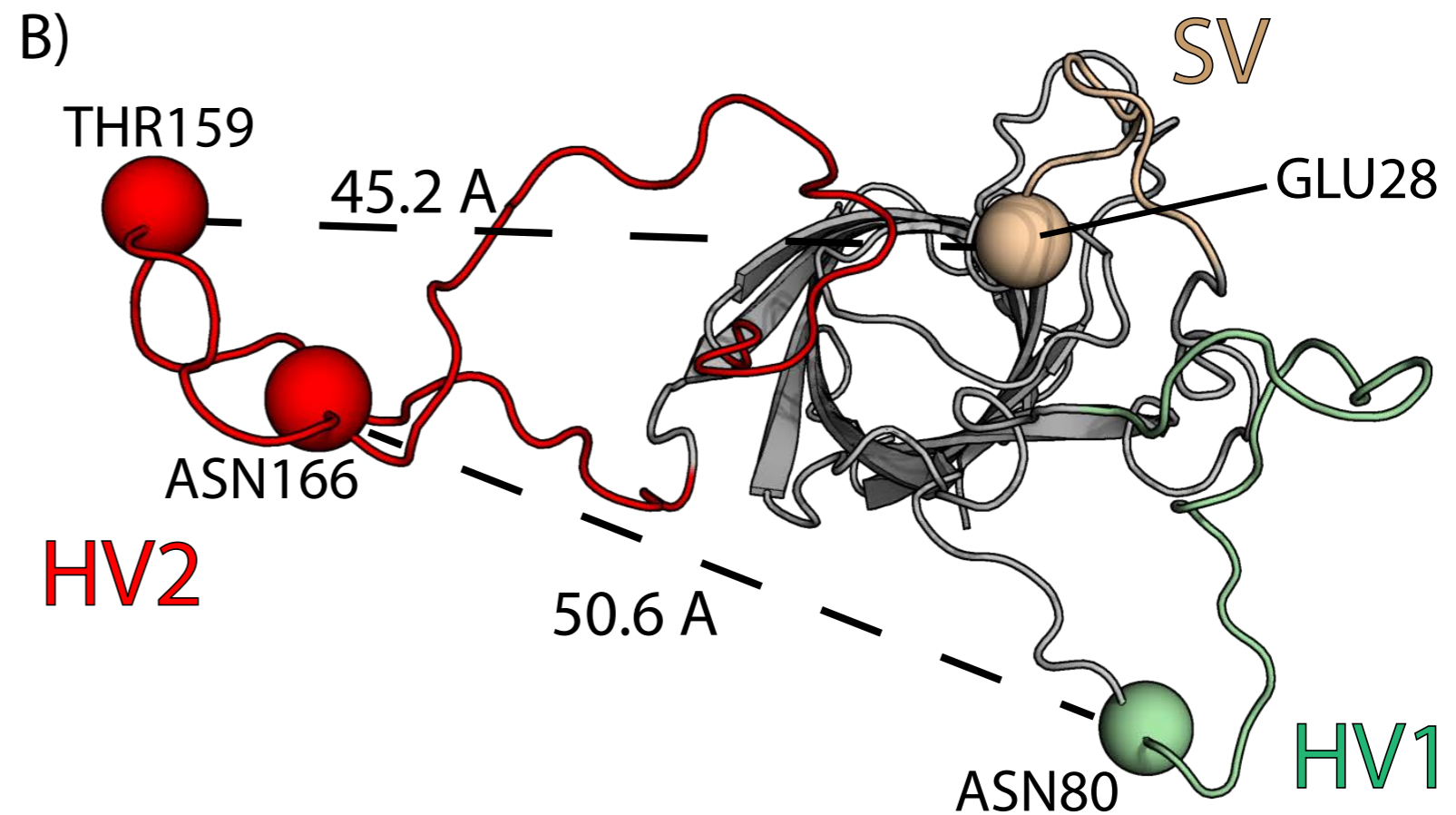
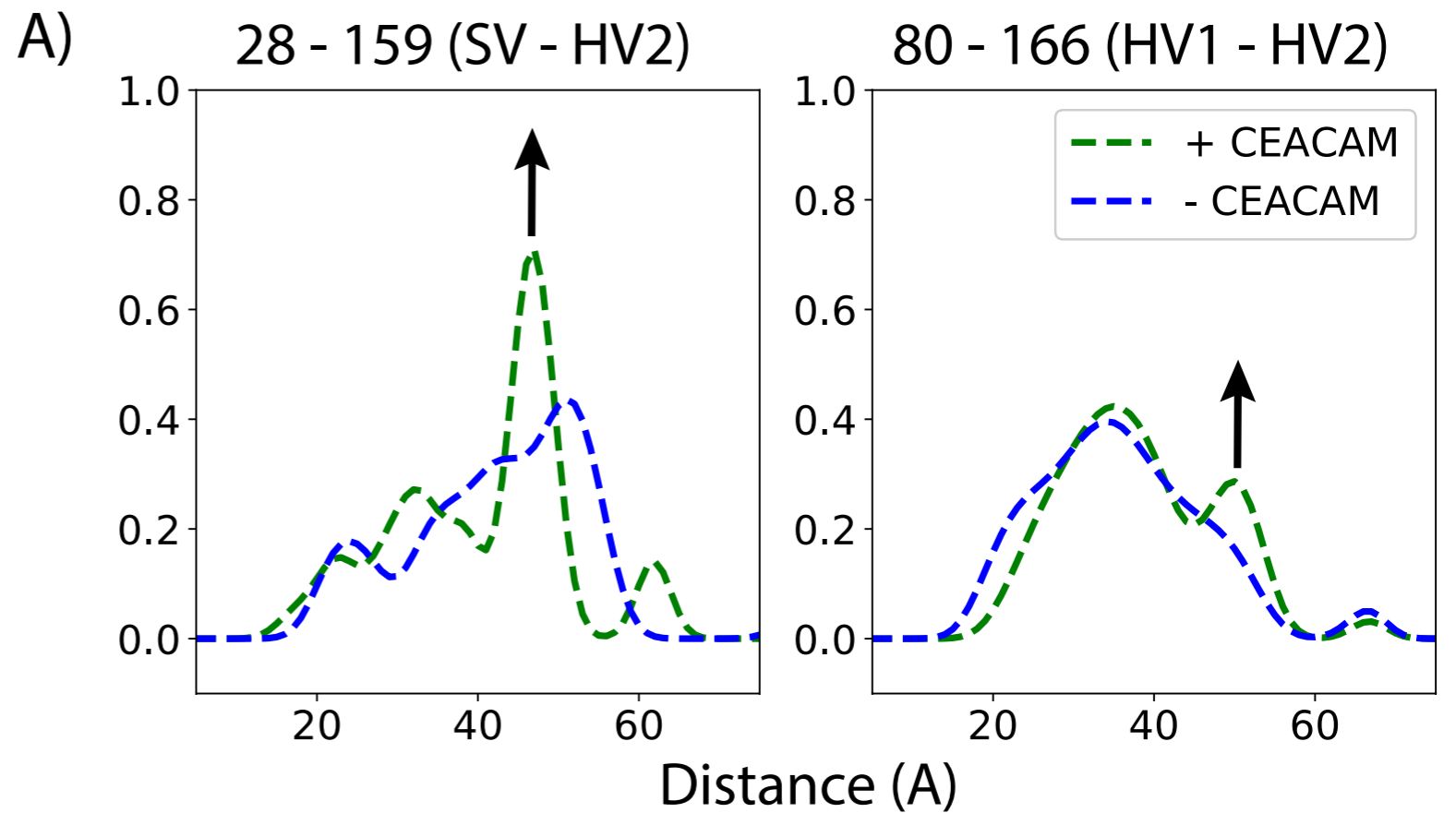
HV2 extended: 20 %



Multiple loop extensions: 40 %

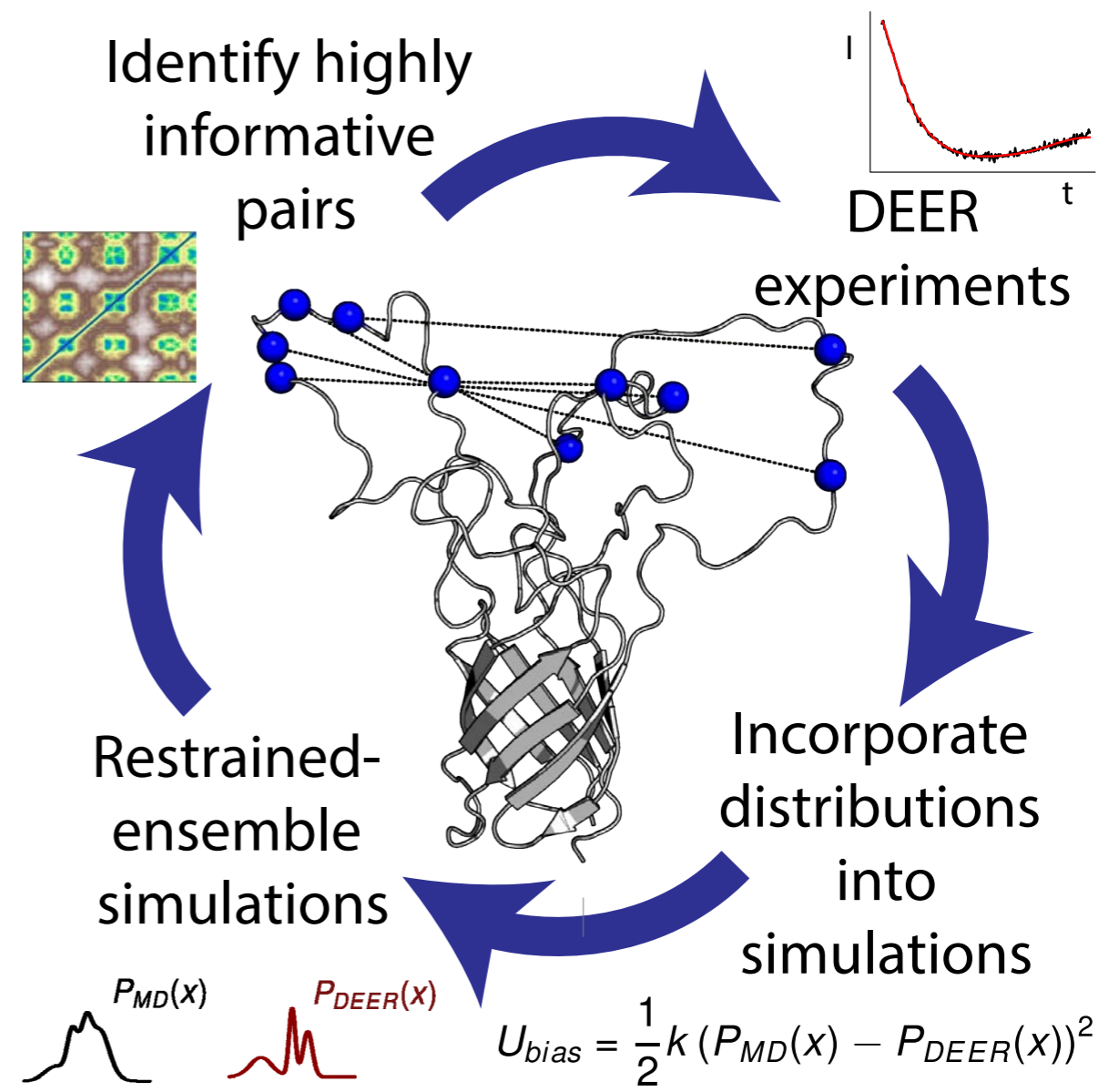


HV2-extended conformations are selected during CEACAM engagement



# Summary

- By incorporating optimally-selected DEER pairs into MD simulation, we have obtained a conformational ensemble of apo Opa that suggests clear possible modes of CEACAM engagement
- Further refinement of the bound ensemble
  - binding experiments to determine which loop(s) CEACAM binds: HV2 or the HV1/SV interface?
  - additional simulations +CEACAM



Thank you  
Questions?

Additional Slides

